IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 920976.90172)

In re Application of:	)	
	)	
Thundat et al.	)	
	)	Group Art Unit: 1753
Serial No.: 10/077,633	)	
	)	Examiner: Brian L. Mutschler
Filed: January 30, 2002	)	
	)	
For: Photoelectrochemical Molecular Comb	)	
	)	

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

AFFIDAVIT OF DR. THOMAS G. THUNDAT
(PURSUANT TO 37 C.F.R. SECTION 1.131)

I, Thomas G. Thundat, residing in Knoxville, TN do hereby declare:

1. I am one of the named co-inventors of this United States Letters Patent Application Serial No. 10/077,633, filed on January 30, 2002, and assigned to UT-Battelle LLC.

2. All of the acts and events described in this Affidavit occurred in the United States.

3. The inventions described in the claims of Patent Application Serial No. 10/077,633 were conceived and reduced to practice cooperatively by the named inventors, Thomas G. Thundat, Thomas L. Ferrell, and Gilbert M. Brown prior to November 1, 2001 (hereinafter the reference date).

4. The invention described and claimed in a Patent Application Serial No. 10/077,633 was described by the inventors in a detailed "Report of Possible Subject Invention" that is dated prior to the reference date. The Report is attached as Exhibit A to this affidavit.

5. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that theses statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 9-10-04

Signed: _____
Thomas G. Thundat

OAK RIDGE NATIONAL LABORATORY
Only
MANAGED BY UT-BATTELLE, LLC
FOR THE U.S. DEPARTMENT OF ENERGY

REPORT OF POSSIBLE SUBJECT INVENTION

ORNL-40 *Electronically fillable in WORD 97 or newer* (04/01/2000)

Intellectual Property Section Use

Disclosure No. _____

DOE S- _____

INSTRUCTIONS: Place pointer over highlighted text to reveal instructions here and throughout this document.

PART I - DESCRIPTION OF THE INVENTION

1. SUBMITTER(s): (First/initial/last)

Thomas G. Thundat, Thomas L. Ferrell, and G.M. Brown

2. TITLE: (10 words maximum)

Photoelectrochemical Molecular Comb

3. BRIEF DESCRIPTION OF THE INVENTION:

A novel technique of separating molecules such as DNA, proteins, and other molecules using a photoelectrochemical method is described. In this method a semiconductor in contact with a thin liquid (buffer solution) layer is used as a substrate. Proper biasing of the solid-liquid interface results in the creation of a depletion layer in the semiconductor. The solution of biomolecules to be separated is placed in the buffer. Using a pulsed light source charge carriers are generated in the depletion layer. The separated charge carriers reach the interface and create a localized photovolatge. Charged molecules moves under this voltage. The distance migrated is proportional to the charge and mass of the biomolecule. As the light source is scanned the molecules moves along with the light beam. By properly adjusting the scan speed different molecules can be separated. Unlike conventional electrophoresis where voltage applied is kV, in this method the voltage applied is around 1V.

4. BACKGROUND: (Problem your invention solves)

Separation of molecules such as proteins is very important in biology and medicine. Gelelectrophoresis is routinely used for separation of biomolecules. In gelelectrophoresis biomolecules are allowed to migrate in a gel matrix that is a few centimeters long By applying potential using metal electrodes at the ends of the gel a filed gradient is created. Biomolecules moves under this field and get separated as a function of distance covered. Conventional electrophoresis requires high-applied voltage (kV range). Since high voltage is involved, heating of the gel happens routinely. Since the molecules are moving under a static field, the speed by which molecules move cannot be

controlled. In addition, it requires tens of minutes to get separation of molecules. Also, since high voltage is involved resulting ohmic heating may cause degradation of samples.

5. DETAILED DESCRIPTION OF THE INVENTION: (How to make and use, method steps, best mode, drawings of all embodiments)

Here we describe a novel technique and apparatus to achieve chemical separation of molecules using electrochemistry. The substrate used in this method is a semiconductor. Ge, GaAs, TiO2, CdS, etc. can be used for this purpose. However, it is important to make sure that the voltage can be applied across the solid liquid interface. This requires cleaning the substrate of thicker insulating oxides. For example, the oxide on the Ge substrate can be cathodically reduced or removed using mild etching solution.

The substrate material is brought in contact with a thin layer of conducting liquid. A thin layer of gel on the substrate can also be used. By applying suitable potential across the interface a depletion region can be created in the semiconductor. This requires an ohmic back contact on the semiconductor. A second electrode placed in the solution serves as the counter electrode. The counter electrode can be a transparent conductor such as indium tin oxide (ITO) with same area as the working electrode, but separated by microns. It is also possible to use a reference electrode placed very close to the substrate for accurate measurement of potential drop across the interface. Since the back contact is ohmic almost all the applied potential will be dropped in the depletion layer. The voltage applied needs to be only a few volts.

If we expose the solid-liquid interface to light with photon energy larger than the band gap, electron-hole pairs are created in the depletion layer in the semiconductor. The field in the depletion layer separates the electrons and holes, which move in opposite directions. By proper choice of semiconductor material (p-type or n-type) and potential (cathodic or anodic), either electrons or holes can be brought to the surface. If a p-type material is used, the electrons come to solid-liquid interface. Proper care should be taken to avoid oxide formation or corrosion of the substrate.

In this invention the light source used produces a line (or point) of intense light. Therefore, the electrons arriving at the interface is very localized. Since we are using a pulsed light source no saturation phenomena will

be occurring. The magnitude eof photovoltage induced will be proportional to the light intensity and the extend of band bending. The extend of band bending can be controlled by adjusting the biasing voltage.

In one embodiment the bias is kept constant. The bias is selected in such a way that the photovoltage is maximum. The solid-liquid interface consists of semiconductor surface in contact with a resistive medium such as a gel. The restive medium can be roughened surface or artificially patterned surface. The resistive medium can also be a thin layer of buffer solution.

Now scanning the light beam makes the molecules migrate in the same direction as the direction of scan. By adjusting the scanning speed it is possible to separate the molecules of different mobilities.

It is also possible to construct arrays of light beams by which a single sweep can separate a large number of molecules such as proteins. It is possible to design arrays by which proteins can be separated and DNA can be sequenced.

In another embodiment the counter electrode is a transparent conductor such as indium tin oxide kept at a micrometer distance above the substrate. The gap between the counter and working electrode is filled with buffer solution or gel. The counter electrode is coated such that the analyte molecules would not deposit on the counter electrode. When a line of instantaneous photopotential is created between the working and counter electrode the analyte molecules moves towards the counter electrode. Since the light beam is scanned the molecules move in a direction almost parallel to the substrate resulting in separation.

In a third embodiment, the applied potential between the counter and reference electrode is alternated in such a way that the photopotential is alternated. This causes the analyte molecular motion to alternate between counter and working electrode. Now scanning the light beam causes the movement of the molecules in a direction parallel to the substrate resulting in separation of the molecules.

We claim:

1) An apparatus for separating molecules at a semiconductor/liquid interface comprising:

A doped semiconductor with a surface in contact with liquid with an ohmic contact in the back, said semiconductor-liquid interface potential is controlled by external power supply to crate a depletion layer at the solid-liquid interface in the semiconductor;

A liquid films where the molecules to be separated can be placed at one end;

A beam of light source illuminating only a narrow band of the solid liquid interface starting at the location of sample molecules;

a light source with energy enough to create electron-hole pairs in the depletion layer with electrons coming to the interface creating a highly localized photopotential at the interface;

a scanning mechanism to scan the light beam along the surface of the semiconductor.

2) An apparatus as described in claim 1 where the said semiconductor material is from a class of semiconductors such as Si, Ge, GaAs, CdS, ZnO or other type of semiconductors or semi-insulators that can be used for creating a depletion layer.

3) An apparatus as described in claim 1 where the said liquid layer has a thickness from nm to micrometers and the said liquid layer is a resistive gel from a group consisting of polymethyl methacralate, agrose, or materials used for gel electrophoresis.

4) An apparatus as described in claim 1 where the light source is a laser with energy greater than the band gap of the semiconductor material.

5) An apparatus as decried in claim 1 where the light source is pulsed, chopped, or modulated.

6) An apparatus as described in claim 1where the incident light on the semiconductor-liquid interface is scanned from the sample side to the opposite side at least once or in plurality.

7) An apparatus as described in claim 1 where the semiconductor-liquid interface is powered with a potetiostat with two or three electrode configuration.

8) An apparatus as described in claim 1 where the semiconductor is moved with respect to the light source.

9) An apparatus as described in claim 1 where the applied potential on the semiconductor-liquid interface is modulated and the light source is unmodulated.

10) An apparatus as described in claim 1 where the semiconductor surface is artificially patterned to provide resistance to the molecular motion for separation.

11) An apparatus for separating biomolecules and biochemicals using a thin layer of resistive liquid in contact with a semiconductor that is noncentrosymmetric that exhibit surface piezoelectricity such as GaAs comprising:

an external electrical power source by which a depletion layers is crated in the semiconductor;

a finely focused line of light to create electron-hole pairs in the depletion layer;

a means to scan the light beam from sample side to opposite side.

12) An apparatus as described in claim 1 where the counter electrode is a transparent electrically conducting surface such as indium tin oxide where the gap between the electrode is filled with solution or gel;

13) An apparatus as described in claim 12 where the applied potential between the electrode is alternated in such a way as to create an alternating pulsed photopotential preventing the analyte molecules from adsorbing or depositing on either electrode.

14) An apparatus as described in claim 12 where the light beam is scanned making the analyte molecules to be separated in time.

6. RELATED TECHNOLOGY: (List all relevant publications, patents, etc. of yours and others, and submit a copy of each with this form.)

This is a unique twist in the electrophoretic separation. No technology resembling this method exists at this time.

7. UNIQUE FEATURES: (List all features that distinguish the invention over the technology listed in Item 6.)

This is a unique method that is capable of performing molecular separation of DNA, proteins etc. The advantages include:

- No high volatge
- Simple and fast operation
- Does not produce heat
- Disposable units can be made
- Can work with diode lasers

8. POSSIBLE ALTERNATIVE VERSIONS:

The device can be used for sequencing DNA, proteins as well as separating other molecules.

9. PROBABLE USES: (Anticipated U.S. Government, Industry, foreign uses of the invention)

Separation of molecules

PART II - FACTS RELATING TO THE INVENTION

10. REDUCTION TO PRACTICE : Select one of the following responses:

- ☐ Invention is purely conceptual and needs experimentation to validate the concept.
- ☐ Invention is conceptual, but does not need experimentation to validate the concept.
- ☒ Proof-of-principle experiment has been performed to validate the concept.
- ☐ Invention has been demonstrated on a [☒ laboratory; ☐ prototype; ☐ production] scale.
- ☐ Other (Explain):

11. SOURCE(S) OF FUNDS: (Funding under which the invention arose)

- ☒ DOE B&R Code: ERKP261
- ☐ 100% funds-in from third party identified below
- ☐ LDRD
- ☐ Seed Money
- ☐ Other:

Identify respective Program Manager:

12. THIRD PARTY: Is a third party involved in the invention? ☐ YES ☒ NO

If yes, provide the following information:

Note: A submitter who is not a UT-Battelle employee is a third party.

- ☐ CRADA
- ☐ Subcontract
- ☐ Interagency Agreement
- ☐ Work For Others
- ☐ No written agreement
- ☐ Other:

Name of third party:

Contract or Agreement No. Effective dates:

Explain any special circumstances:

13. SUBMITTERS: Each submitter must provide all the following information and original signature.

A. Full Name Thomas G. Thundat SS#: 057-66-5022 Citizenship: USA

Residence Address: 616 Plainfield Rd., Knoxville, TN Telephone: 865-691-4638

Current Employer: ☒*UT-Battelle* ☐Other:

Employee No.: 34530 Work Address: ORNL, 4500S,G-148, MS-6123 Telephone: : 865-574-6201

DIVISION No.: 1 Name: LSD Manager: R.C. Mann Supervisor: J.C. Miller

My specific contribution to the concept of the invention is:

Conceived the original concept of molecular separation using photovoltage technique

Recorded in Notebook # Page(s) Date of Entry* Witnessed by:

Employer on *Date of Entry: ☒*UT-Battelle* ☐Other:

DIVISION No.: 01 Name: LSD Manager: R.C. Mann Supervisor: J.C. Miller

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document: Signature: _____ Date: _____

B. Full Name Thomas L. Ferrell SS#: 403-58-1444 Citizenship: USA

Residence Address: 1600 Hickory Trail Knoxville, TN 37932 Telephone: 865-966-2309

Current Employer: ☒*UT-Battelle* ☐Other:

Employee No.: 20867 Work Address: 4500S, MS-6123 Telephone: : 865-574-6214

DIVISION No.: 01 Name: LSD *Manager:* R.C. MANN *Supervisor:* J.C. MILLER

My specific contribution to the concept of the invention is: co-proposed optical beam techniques

Recorded in Notebook # Page(s) Date of Entry* Witnessed by:

Employer on *Date of Entry: ☒*UT-Battelle* ☐Other:

DIVISION No.: 1 Name: Life Science Manager: R.C. Mann Supervisor: J.C. Miller

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document: Signature: _____ Date: _____

C. Full Name G.M. Brown SS#: 249-82-0312 Citizenship: USA

Residence Address: 1306 Kensigton Dr., Knoxville 37922 Telephone: 865-690-5180

Current Employer: ☒*UT-Battelle* ☐Other:

Employee No.: 20630 Work Address: 4500S, MS6119 Telephone: : 865-576-2756

DIVISION No.: 60 Name: CASD Manager: M. Buchanan Supervisor: B. Moyer

My specific contribution to the concept of the invention is: Co-proposed Electrochemical techniques

Recorded in Notebook # Page(s) Date of Entry* Witnessed by:

Employer on *Date of Entry: ☐*UT-Battelle* ☐Other:

DIVISION No.: Name: Manager: Supervisor:

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document: Signature: Gilbert M Brown Date: / /

D. Full Name SS#: - - Citizenship:

Residence Address: Telephone: - -

Current Employer: ☐*UT-Battelle* ☐Other:

Employee No.: Work Address: Telephone: : - -

DIVISION No.: Name: Manager: Supervisor:

My specific contribution to the concept of the invention is:

Recorded in Notebook # Page(s) Date of Entry* Witnessed by:

Employer on *Date of Entry: ☐*UT-Battelle* ☐Other:

DIVISION No.: Name: Manager: Supervisor:

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document: Signature: _____ Date: _____

E Full Name SS#: - - Citizenship:

Residence Address: Telephone: - -

Current Employer: ☐*UT-Battelle* ☐Other:

Employee No.: Work Address: Telephone: : - -

DIVISION No.: *Name:* *Manager:* *Supervisor:*

My specific contribution to the concept of the invention is:

Recorded in Notebook # Page(s) Date of Entry* Witnessed by:

Employer on *Date of Entry: ☐*UT-Battelle* ☐Other:

DIVISION No.: *Name:* *Manager:* *Supervisor:*

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document: Signature: _____ Date: _____

F. Full Name SS#: - - Citizenship:

Residence Address: Telephone: - -

Current Employer: ☐*UT-Battelle* ☐Other:

Employee No.: Work Address: Telephone: : - -

DIVISION No.: *Name:* *Manager:* *Supervisor:*

My specific contribution to the concept of the invention is:

Recorded in Notebook # Page(s) Date of Entry* Witnessed by:

Employer on *Date of Entry: ☐*UT-Battelle* ☐Other:

DIVISION No.: *Name:* *Manager:* *Supervisor:*

If Other, explain relationship to UT-Battelle and provide a copy of agreement, subcontract, or other documentation:

I have read and understood the contents of this document: Signature: _____ Date: _____

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14. NOTEBOOK RECORDS : All items must be accurately completed. An inventor cannot be a witness.

EVENT	DATE	NOTEBOOK NO.	PAGES	TWO NOTEBOOK WITNESSES	WITNESS DATES
Original Concept		A108383-G		1. Randy James 2. L.A. Pinnad uwage	
First Sketch or Drawing				1. Randy James 2. L.A. Pinnaduwage	
First Written Description				1. Randy James 2. L.A. Pinnaduwage	
First Model or Test Unit				1. 2.	
First Test of Invention				1. 2.	

List any other permanent records of the invention:

Please submit with this form copies of notebook entries and other records and reports relating to the invention. These documents may be essential in determining inventorship and date of the invention.

15. PUBLICATION STATUS: Has the invention been disclosed to the public or any party outside DOE and UT-Battelle?

☐YES ☒NO

If yes, provide the following information:

Was the disclosure cleared through the Technical Information Office? ☐YES (attach copy of clearance form) ☐NO

Indicate the form(s) of the disclosure: ☐Oral ☐Visuals ☐Abstract ☐Full Article ☐Other

☐Submitted for review, but not yet published

Date of Conference or Publication: Location of Conference: Journal:

Other relevant information

16. ROUTINE USE OF THE INVENTION:

If the inventors have tested any embodiment of the invention, has there been any additional, routine use of the invention?

☐YES ☐NO

If yes indicate date and circumstances of first such use:

CONFIDENTIAL INFORMATION

This document contains patentable subject matter and is disclosed in confidence by UT-Battelle, LLC under 35 USC § 205.

PART III - MANAGEMENT REVIEW: (To be completed by submitter's supervisor, division management, or program manager)

17. Invention Achievement: This invention represents a
☒Pioneer Breakthrough ☐Major Improvement ☐Minor Improvement

18. Claims and Enforceability: (check all that apply)

The invention is a:	☒Method	☒Product	☐Machine	☐Composition of Matter
Is the invention detectable in a product?	☒Yes	☐Somewhat	☐No (can be practiced in secret)	
Scope of the invention is:	☒Broad	☐Intermediate	☐Narrow	
Discovery by another is likely in:	☐1 year	☒3 years	☐5 years	
"Inventing around" the invention would be:	☐Easy	☐Moderate	☒Difficult	

19. Quality of Description:
☒Description, documentation, data, drawings, etc. are complete.
☐Description is reasonably complete.
☐Further information is needed to support a patent application.

20. Potential Use of the Invention:
☐U.S. Government only ☐Manufacturer ☐Consumer
☒U.S.A. Companies: Nanogen
Products:
☐Foreign Countries:
Companies:

21. Market Value of the Invention:
Estimated total U.S. market: Present: $10M 5 years $50M
Estimated total world market: Present: $20M 5 years $100M
Use by others (1-10) + Near-term potential (1-10) + Value to related invention disclosures (1-10) = Total (3-30)

(8 + 9 + 8) = 25

22. Recommended Disposition:
☒UT-Battelle should elect and file patent application. ☐Program or division will finance patent application.
☐DOE should file patent application. ☐Do not file a patent application.

23. Reviewer Comments:

Reviewer Name, printed or typed: JOHN C. MILLER Position: SECTION HEAD

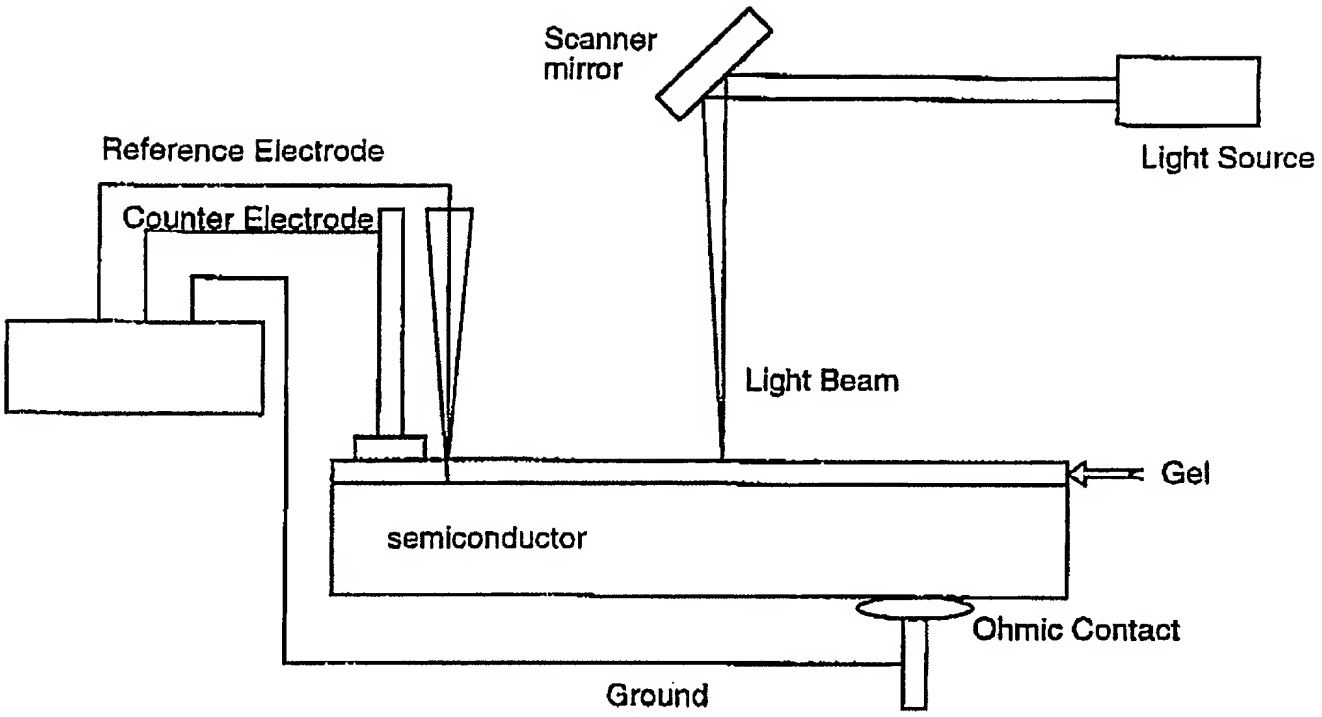
Reviewer Signature: John C. Miller Date: 1/1/

24. CLASSIFICATION: (To be completed by classification officer, or derivative classifier if not DUSA)
This document is properly classified as:
☐Confidential ☐Secret ☐Restricted Data ☐Formerly Restricted Data ☐National Security Information
☐Unclassified (Contains no classified information) ☐DUSA (no signature required)

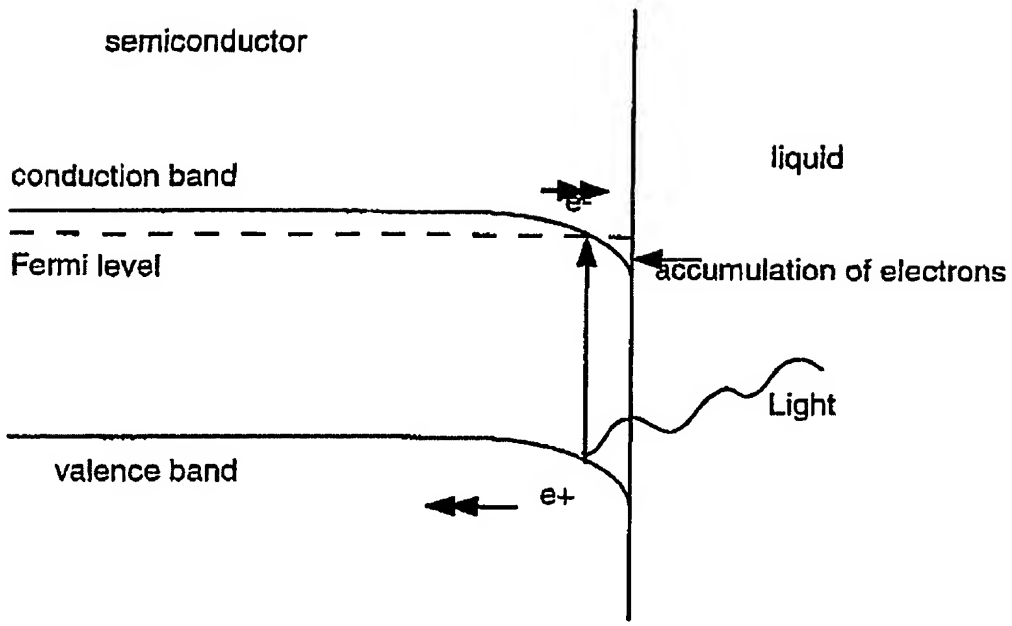
Signature: _____ Date: _____

25. RECEIPT BY INTELLECTUAL PROPERTY SECTION: *(This form must be complete in order to be accepted.)*

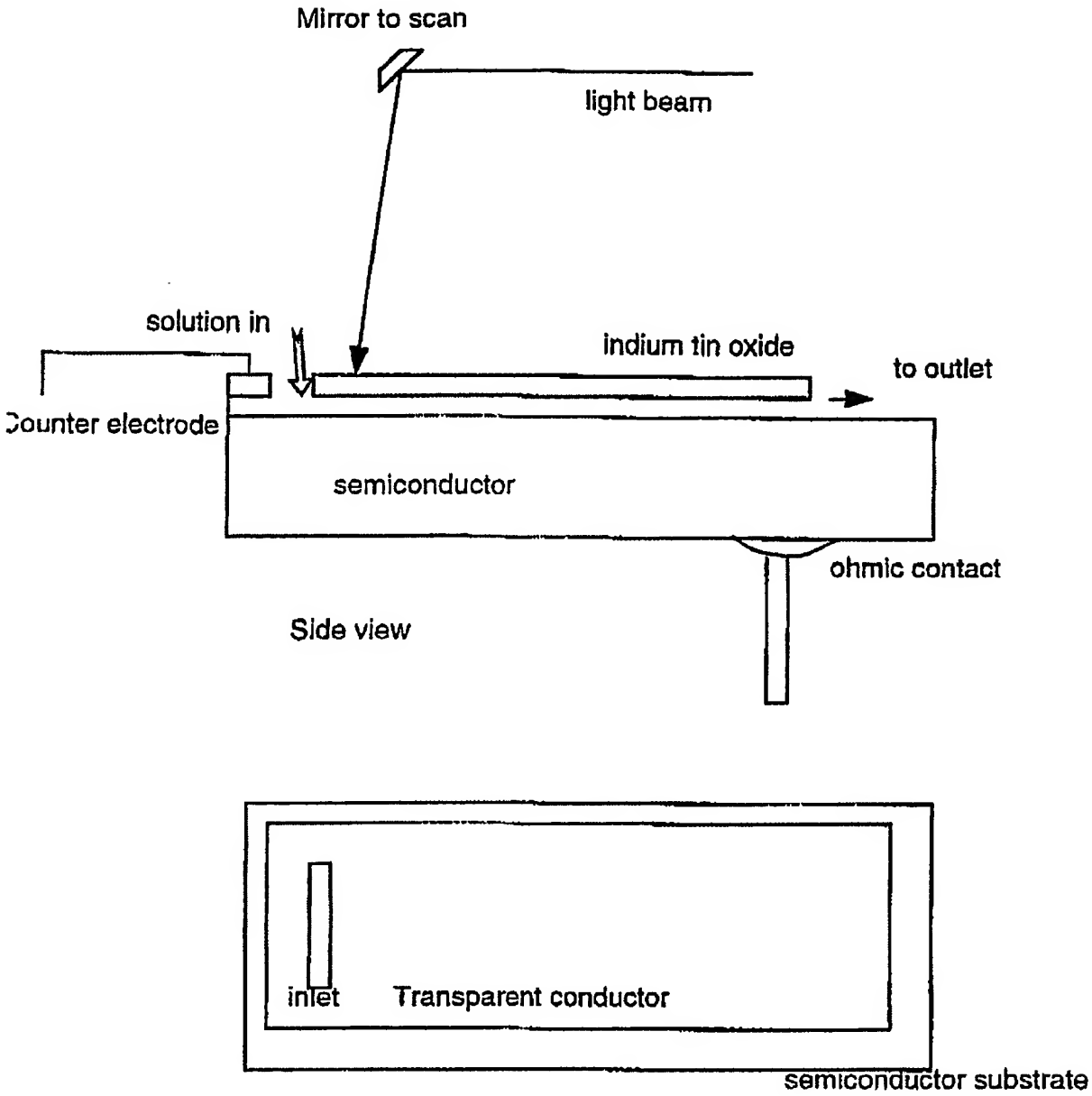
Received by: _____ Date: _____



Experimental arrangement



Energy band diagram


Mirror to scan
light beam
solution in
indium tin oxide
to outlet
Counter electrode
semiconductor
ohmic contact
Side view
inlet
Transparent conductor
semiconductor substrate
Top view

Patterned semiconductor surface